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INVESTIGATION OF THE PROLONGED RECOVERY TIME  
FOLLOWING BOTULINUM INTOXICATION - EFFICACY OF  
INTRAPERITONEAL VERSUS INTRAVENOUS INJECTION  
OF BOTULINUM ANTITOXIN

FINAL REPORT

by

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EFFICACY OF INTRAPERITONEAL VERSUS INTRA-  
VENOUS INJECTION OF BOTULINUM ANTITOXIN

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| <p>Studies to provide information concerning botulinum intoxication were conducted in an attempt to determine what parameters influence the prolonged recovery time following intoxication and if the routes of antitoxin treatment could influence the rates of recovery and decrease mortality in test species. (U)</p> <p>In order to study prolonged recovery times, mice were injected via the intramuscular route with sublethal concentrations of botulinum type A toxin. Proteolytic enzyme solutions were injected into the test site to determine if the removal of bound proteinacious toxin from neuromuscular junctions could influence paralytic recovery times. Parameters of observation were gait of animal, production of lesion at site of injection and activity of paralyzed and treated mice. Trypsin and protease at 1 mg concentrations appeared to markedly influence recovery whereas chymotrypsin, papain, ficin, pepsin and CaCl<sub>2</sub> did not significantly affect decrease of paralysis. (U)</p> <p>The efficacy of intraperitoneal versus intravenous injection of botulinum antitoxin was studied by initiating a state of paralysis with resulting death of groups of mice and prophylactically administering specific antitoxin by either the intravenous or intraperitoneal routes. There appeared to be no effect of route of antitoxin administration on protective action of antisera therapy. (U)</p> |  |   |                 |

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|     |  | ROLE   | WT | ROLE   | WT | ROLE   | WT |
|     |  |        |    |        |    |        |    |
|     | Type A Cl. botulinum toxin<br>Botulinum paralysis<br>Botulinum therapy<br>Enzyme therapy<br>Botulinum prophylaxis<br>Swiss-Webster mice<br>Fort Detrick Inbreed mice |        |    |        |    |        |    |

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INVESTIGATION OF THE PROLONGED RECOVERY TIME  
FOLLOWING BOTULINUM INTOXICATION - EFFICACY OF  
INTRAPERITONEAL VERSUS INTRAVENOUS INJECTION  
OF BOTULINUM ANTITOXIN

SUMMARY

Studies to provide information concerning botulinum intoxication were conducted in an attempt to determine what parameters influence the prolonged recovery time following intoxication and if the routes of antitoxin treatment could influence the rates of recovery and decrease mortality in test species.

In order to study prolonged recovery times, mice were injected via the intramuscular route with sublethal concentrations of botulinum type A toxin. Proteolytic enzyme solutions were injected into the test site to determine if the removal of bound proteinaceous toxin from neuromuscular junctions could influence paralytic recovery times. Parameters of observation were gait of animal, production of lesion at site of injection and activity of paralyzed and treated mice. Trypsin and protease at 1 mg concentrations appeared to markedly influence recovery whereas chymotrypsin, papain, ficin, pepsin and  $\text{CaCl}_2$  did not significantly affect decrease of paralysis.

The efficacy of intraperitoneal versus intravenous injection of botulinum antitoxin was studied by initiating a state of paralysis with resulting death of groups of mice and prophylactically administering specific antitoxin by either the intravenous or intraperitoneal routes. There appeared to be no effect of route of antitoxin administration on protective action of antisera therapy.

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### INVESTIGATION OF THE PROLONGED RECOVERY TIME FOLLOWING BOTULINUM INTOXICATION

The course of botulinal intoxication following the binding of toxin to a specific site in the nerve fiber was studied by injecting mice with a sublethal paralyzing dose of botulinum type A toxin. Toxin which was not bound immediately following injection was neutralized intraperitoneal inoculation with specific antitoxin.

As reported in previous reports, testing criteria to determine the extent and severity of paralysis consisted of use of test mouse motion in an activity cage. As the mouse moves through beams of invisible light, activity is measured as counts per period of time.

The results of activity of mice following administration of toxin by the intramuscular, intraperitoneal and stomach intubation routes are presented in Table 1. Significant paralysis was observed following intramuscular injection of  $1/2$  LD<sub>50</sub> of toxin.

Following these initial experiments, tests were performed to determine if injection of various proteolytic enzymes could destroy botulinum toxin in vivo. In the previous experiments four mice were placed in the activity cage. Three mice per group were used in these experiments. One group of three received either two LD<sub>50</sub> of toxin in the left hind leg or PBS diluent. One hour later all animals received 0.1 ml of antitoxin and four hours later all animals received 0.1 ml of freshly prepared proteolytic enzyme containing 10 mg/ml in the

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TABLE 1  
ACTIVITY OF MICE FOLLOWING ADMINISTRATION  
OF DIFFERENT AMOUNTS OF TOXIN - ACTIVITY  
PRESENTED AS PERCENT OF CONTROL GROUP

| Route of<br>Injection | Percent<br>LD <sub>50</sub> | Day    |        |        |        |
|-----------------------|-----------------------------|--------|--------|--------|--------|
|                       |                             | 1      | 4      | 11     | 15     |
| Intra-<br>muscular    | 1/2                         | 45.96  | 34.16  | 64.91  | 82.48  |
|                       | 1/4                         | 82.72  | 89.11  | 107.45 | 88.73  |
|                       | 1/8                         | 94.68  | 91.62  | 101.94 | 89.18  |
| Intraperi-<br>toneal  | 1/2                         | 86.99  | 191.10 | 105.11 | 111.06 |
|                       | 1/4                         | 285.11 | 436.84 | 159.56 | 158.07 |
|                       | 1/8                         | 162.58 | 284.47 | 142.56 | 187.63 |
| Stomach<br>Intubation | 1/2                         | 29.84  | 12.20  | 106.79 | 137.62 |
|                       | 1/4                         | 67.91  | 119.75 | 94.93  | 129.00 |
|                       | 1/8                         | 90.97  | 136.03 | 173.28 | 158.79 |

same hind leg. Chymotrypsin, protease, trypsin and  $\text{CaCl}_2$  (control) were used as saline solutions. The animals were observed for walk and stance, ability to walk down a vertical rod, and presence of enzyme-induced lesion or sore at the site of injection for two weeks. The results are presented in Tables 2, 3 and 4.

Tables 5 and 6 present activity cage results of mice treated with toxin to induce paralysis and then injected with proteolytic enzymes. This data demonstrated marked paralysis in all toxin-treated animals except those receiving protease and trypsin. Papain and ficin resulted in extensive tissue damage at the site of injection.

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TABLE 2

VERTICAL ROD, GAIT AND STANCE, AND SORES AT THE  
INJECTION SITE AS A RESULT OF ENZYME TREATMENT

| Treatment | Toxin Animals |    |   |    |    | Diluent Animals |   |    |    |    |
|-----------|---------------|----|---|----|----|-----------------|---|----|----|----|
|           | Day           |    |   |    |    | Day             |   |    |    |    |
|           | 1             | 3  | 7 | 11 | 16 | 1               | 3 | 7  | 11 | 16 |
| Control   |               |    |   |    |    |                 |   |    |    |    |
| Vert      |               |    | + | ++ | +  |                 |   |    |    | +  |
| Gait      |               |    |   |    |    |                 |   |    |    |    |
| Sore      |               |    |   |    |    |                 |   |    |    |    |
| Vert      |               | +  | + | ++ | +  |                 |   |    |    |    |
| Gait      | +             |    |   |    |    |                 |   |    |    |    |
| Sore      |               |    |   |    |    |                 |   |    |    |    |
| Vert      |               |    | + |    |    |                 |   |    |    |    |
| Gait      | +             |    | + |    |    |                 |   |    |    |    |
| Sore      |               |    |   |    |    |                 |   |    |    |    |
| Pepsin    |               |    |   |    |    |                 |   |    |    |    |
| Vert      | +             | ++ | + |    |    |                 |   |    |    |    |
| Gait      | ++            | +  |   |    |    |                 |   |    |    |    |
| Sore      |               |    |   |    |    |                 |   |    |    |    |
| Vert      |               | +  | + | +  |    |                 |   |    |    |    |
| Gait      | +             | +  | + |    |    |                 |   |    |    | +  |
| Sore      |               |    |   |    |    |                 |   |    |    |    |
| Vert      |               | +  | + | +  |    |                 |   |    |    |    |
| Gait      | +             | +  |   |    |    |                 |   |    |    |    |
| Sore      |               |    |   |    |    |                 |   |    |    |    |
| Trypsin   |               |    |   |    |    |                 |   |    |    |    |
| Vert      |               | +  | + |    |    |                 |   |    |    |    |
| Gait      | +             |    | + |    |    |                 |   |    |    | +  |
| Sore      | +             |    | + |    |    |                 |   |    |    | +  |
| Vert      | +             | +  | + | +  | ++ |                 |   |    |    |    |
| Gait      | +             |    | + |    |    |                 |   |    |    | ++ |
| Sore      |               |    |   |    |    |                 |   |    |    |    |
| Vert      |               | ++ | + | ±  |    |                 |   |    |    |    |
| Gait      | +             | +  | + |    |    |                 |   | ++ |    | +  |
| Sore      |               |    | + |    |    |                 |   |    |    | +  |

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TABLE 2 (Contd)

VERTICAL ROD, GAIT AND STANCE, AND SORES AT THE  
INJECTION SITE AS A RESULT OF ENZYME TREATMENT

| Treatment             | Toxin Animals |     |    |    |    | Diluent Animals |     |   |    |    |
|-----------------------|---------------|-----|----|----|----|-----------------|-----|---|----|----|
|                       | Day           |     |    |    |    | Day             |     |   |    |    |
|                       | 1             | 3   | 7  | 11 | 16 | 1               | 3   | 7 | 11 | 16 |
| <b>α Chymotrypsin</b> |               |     |    |    |    |                 |     |   |    |    |
| Vert                  |               |     |    | +  |    |                 |     |   |    |    |
| Gait                  | +             |     |    |    |    | +               |     |   |    |    |
| Sore                  | +             |     | +  |    |    | +               |     | + |    |    |
| Vert                  |               |     |    |    |    |                 |     |   |    |    |
| Gait                  |               |     |    |    |    | +               |     |   |    |    |
| Sore                  | +             |     | +  |    |    |                 |     |   |    |    |
| Vert                  |               |     |    | +  |    |                 |     |   |    |    |
| Gait                  | +             |     |    |    |    | +               |     |   |    |    |
| Sore                  | +             |     | +  |    |    |                 |     |   |    |    |
| <b>Papain</b>         |               |     |    |    |    |                 |     |   |    |    |
| Vert                  |               | ++  | +  | +  |    | +               | +++ | * |    |    |
| Gait                  | ++            | +   | +  |    |    | +++             | +++ |   |    |    |
| Sore                  | +             |     | +  |    |    | ++              | ++  |   |    |    |
| Vert                  |               |     |    |    |    | +               |     |   |    |    |
| Gait                  | +             |     |    |    |    | +               |     |   |    |    |
| Sore                  | +             |     |    |    |    | +               |     |   |    |    |
| Vert                  | +             | ++  |    | +  | +  |                 |     |   |    |    |
| Gait                  | ++            | +   |    |    |    |                 |     |   |    |    |
| Sore                  | +             |     | +  |    |    |                 |     |   | +  |    |
| <b>Ficin</b>          |               |     |    |    |    |                 |     |   |    |    |
| Vert                  |               | +++ | +  | +  | +  |                 | +   |   |    |    |
| Gait                  | ++            | ++  |    |    |    | +               |     |   |    |    |
| Sore                  | +             |     | +  | +  |    | +               |     |   | +  |    |
| Vert                  | +             | +   | +  | +  | +  |                 | ++  |   |    |    |
| Gait                  | ++            |     |    |    |    | +               | +   |   |    |    |
| Sore                  | +             |     | +  | +  |    | +               |     |   | +  | +  |
| Vert                  |               | +   |    | +  | +  |                 | +++ | + | +  | +  |
| Gait                  | ++            | +   | ++ |    |    | +++             | +++ | + |    |    |
| Sore                  | +             |     | +  | +  |    | +               | +   | + | +  |    |

\* Leg badly crippled

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TABLE 2 (Contd)

VERTICAL ROD, GAIT AND STANCE, AND SORES AT THE  
INJECTION SITE AS A RESULT OF ENZYME TREATMENT

[illegible]

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TABLE 3

VERTICAL ROD, GAIT AND STANCE, AND SORES AT THE  
INJECTION SITE AS A RESULT OF ENZYME TREATMENT

NEOSTIGMINE INDUCED HYPERSENSITIVITY

| Treatment          | Toxin Animals |      |      | Diluent Animals |      |      |
|--------------------|---------------|------|------|-----------------|------|------|
|                    | Vert          | Gait | Sore | Vert            | Gait | Sore |
| Control            | +             | +    | +    | -               | -    | -    |
| Pepsin             | +             | +    |      | -               |      |      |
| Trypsin            | -             |      |      |                 |      |      |
| α Chymotrypsin     | -             | -    |      | -               |      |      |
| Papain             | +             | -    |      | -               |      |      |
| Ficin              | -             |      |      |                 |      |      |
| Protease           | -             | -    |      | -               |      |      |
| CaCl <sub>2</sub>  | +             | -    |      |                 |      |      |
| Uninjected Control | -             |      |      |                 |      |      |

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VERTICAL ROD, GAIT AND STANCE, AND SORES AT THE  
INJECTION SITE AS A RESULT OF ENZYME TREATMENT

RELATIVE TO ENZYME CONCENTRATION

[illegible]

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TABLE 4 (Contd)

VERTICAL ROD, GAIT AND STANCE, AND SORES AT THE  
INJECTION SITE AS A RESULT OF ENZYME TREATMENT  
RELATIVE TO ENZYME CONCENTRATION

[illegible]

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TABLE 4 (Cont'd)

VERTICAL ROD, GAIT AND STANCE, AND SORES AT THE  
INJECTION SITE AS A RESULT OF ENZYME TREATMENT

RELATIVE TO ENZYME CONCENTRATION

[illegible]

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TABLE 5  
PERCENT ACTIVITIES OF MICE PARALYZED  
WITH BOTULINUM TOXIN AND TREATED  
PROPHYLACTICALLY WITH PROTEOLYTIC ENZYMES

| Treatment         | Day  |       |       |       |       |
|-------------------|------|-------|-------|-------|-------|
|                   | 1    | 3     | 7     | 11    | 16    |
| Control           | 50.1 | 48.6  | 73.1  | 68.1  | 49.4  |
| Pepsin            | 37.2 | 41.2  | 46.1  | 39.1  | 53.0  |
| Trypsin           | 88.1 | 80.4  | 81.9  | 86.2  | 115.8 |
| Chymotrypsin      | 54.4 | 54.7  | 85.9  | 61.0  | 79.9  |
| Papain            | 43.2 | 38.9  | 46.6  | 51.0  | 52.8  |
| Ficin             | 74.6 | 57.0  | 68.8  | 86.2  | 71.5  |
| Protease          | 66.8 | 121.8 | 110.7 | 121.4 | 94.2  |
| CaCl <sub>2</sub> | 43.2 | 33.9  | 51.5  | 53.1  | 57.5  |

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TABLE 6  
PERCENT ACTIVITIES OF MICE TREATED WITH  
DIFFERENT CONCENTRATIONS OF PROTEOLYTIC ENZYMES  
FOLLOWING BOTULINAL PARALYSIS

| Treatment         | Dosage<br>Level<br>mg | Percent Decrease of Control |       |       |       |       |
|-------------------|-----------------------|-----------------------------|-------|-------|-------|-------|
|                   |                       | Day                         |       |       |       |       |
|                   |                       | 1                           | 4     | 7     | 11    | 18    |
| Control           | -                     | 61.6                        | 63.8  | 80.7  | 82.5  | 78.2  |
| Chymotrypsin      | 1                     | 55.0                        | 77.0  | 72.8  | 50.5  | 54.3  |
| CaCl <sub>2</sub> | 1                     | 91.8                        | 188.3 | 236.1 | 179.2 | 177.3 |
| Protease          | 1                     | 62.6                        | 54.4  | 90.9  | 84.1  | 90.4  |
| Protease          | 0.33                  | 109.4                       | 89.9  | 222.4 | 210.9 | 184.4 |
| Protease          | 0.11                  | 52.9                        | 73.7  | 73.8  | 56.4  | 79.2  |
| Trypsin           | 1                     | 49.2                        | 51.1  | 75.3  | 52.4  | 62.3  |
| Trypsin           | 0.33                  | 144.5                       | 245.9 | 591.7 | 380.7 | 292.3 |
| Trypsin           | 0.11                  | 50.7                        | 81.3  | 47.6  | 76.3  | 87.3  |

EFFICACY OF INTRAPERITONEAL VERSUS INTRAVENOUS INJECTION  
OF BOTULINUM ANTITOXIN

Studies conducted were designed to compare the difference in efficacy between intraperitoneal (IP) and intravenous (IV) injection of botulinum antitoxin following stomach intubation of type A botulinum toxin into Swiss-Webster mice. Mice were inoculated with 0.25 ml of toxin solution by the per os route by inserting a round end needle into the stomach of each mouse and expressing the toxin. Botulinum antitoxin (Wellcome Lot K 9710) was serially diluted in saline and 0.1 ml of each dilution was injected either by the IP or IV route into each toxin treated mouse. The results of initial studies are presented in Table 7. Marked differences in protection were not noted.

A second group of experiments was conducted in which greater dilutions of antitoxin were used in order to determine if the low death rates initially observed were due to high antitoxin concentrations. Again, a significant difference between the two routes was not observed. This data is presented in Table 8. In addition, species sex did not alter the results which were obtained as shown in Table 9.

A chance observation within the laboratory indicated mice which are fasted become more susceptible to botulinal intoxication. Table 10 presents data obtained when mice were fasted for 24 hours and inoculated with 10-fold dilutions of botulinum type A toxin by stomach intubation. A total of three trials was conducted. Two of three trials provides expected dose-response results.

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TABLE 7

INTRAPERITONEAL VERSUS INTRAVENOUS PROPHYLAXIS  
OF BOTULINAL INTOXICATION IN MICE

| Route of<br>Antitoxin<br>Admn. | Dilution<br>of<br>Antitoxin | Number of Deaths<br>Post Injection (Hours) |    |    |    |    |    |    | Dead/Total |
|--------------------------------|-----------------------------|--|----|----|----|----|----|----|------------|
|                                |                             | 7  | 23 | 31 | 48 | 56 | 72 | 80 |            |
| IV                             | $10^{-4}$                   | 1  | 4  | 1  | 1  | 0  | 0  | 0  | 7/20       |
|                                | $10^{-3}$                   | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 1/20       |
|                                | $10^{-2}$                   | 1  | 1  | 0  | 0  | 0  | 0  | 0  | 2/20       |
|                                | $10^{-1}$                   | 1  | 1  | 0  | 0  | 0  | 1  | 0  | 3/20       |
| IP                             | $10^{-4}$                   | 0  | 3  | 0  | 0  | 0  | 0  | 0  | 3/20       |
|                                | $10^{-3}$                   | 2  | 1  | 0  | 0  | 0  | 0  | 0  | 3/20       |
|                                | $10^{-2}$                   | 1  | 0  | 0  | 1  | 0  | 0  | 0  | 2/20       |
|                                | $10^{-1}$                   | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0/20       |

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TABLE 8

INTRAPERITONEAL VERSUS INTRAVENOUS PROPHYLAXIS  
OF BOTULINAL INTOXICATION IN MICE  
USING DILUTE CONCENTRATIONS OF ANTITOXIN

| Route of<br>Antitoxin<br>Admn. | Dilution<br>of<br>Antitoxin | Number of Deaths<br>Post Injection (Hours) |    |    |    |    |    |    |    |     | Dead/<br>Total |
|--------------------------------|-----------------------------|--|----|----|----|----|----|----|----|-----|----------------|
|                                |                             | 4  | 20 | 28 | 44 | 52 | 68 | 76 | 92 | 106 |                |
| IV                             | $10^{-5}$                   | 0  | 1  | 1  | 0  | 0  | 0  | 0  | 2  | 0   | 4/20           |
|                                | $10^{-4}$                   | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0   | 0/20           |
|                                | $10^{-3}$                   | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0   | 1/20           |
|                                | $10^{-2}$                   | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0   | 1/20           |
| IP                             | $10^{-5}$                   | 0  | 2  | 0  | 0  | 0  | 0  | 0  | 0  | 0   | 2/20           |
|                                | $10^{-4}$                   | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0   | 0/20           |
|                                | $10^{-3}$                   | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0   | 0/20           |
|                                | $10^{-2}$                   | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0   | 0/20           |
| Control                        | -                           | 0  | 1  | 0  | 1  | 0  | 0  | 0  | 0  | 0   | 2/20           |

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TABLE 9  
THE EFFECT OF SEX ON THE INTRAPERITONEAL  
VERSUS INTRAVENOUS PROPHYLAXIS  
OF BOTULINAL INTOXICATION IN MICE

| Route of<br>Antitoxin<br>Admn. | Dilution<br>of<br>Antitoxin | Sex   | Number of Deaths<br>Post Inj. (Hours) |    |    |    |    | Dead/Total |
|--------------------------------|-----------------------------|-------|---------------------------------------|----|----|----|----|------------|
|                                |                             |       | 8                                     | 24 | 32 | 48 | 96 |            |
| IV                             | 10 <sup>-5</sup>            | 20M*  | 0                                     | 1  | 0  | 3  | 0  | 4/20       |
|                                | 10 <sup>-4</sup>            | 20M   | 0                                     | 0  | 0  | 0  | 0  | 0/20       |
|                                | 10 <sup>-3</sup>            | 10M   | 0                                     | 1  | 0  | 0  | 0  | 1/10       |
|                                |                             | 10F** | 0                                     | 0  | 0  | 0  | 0  | 0/10       |
|                                | 10 <sup>-2</sup>            | 10M   | 0                                     | 1  | 0  | 0  | 1  | 2/10       |
|                                |                             | 10F   | 0                                     | 0  | 0  | 1  | 0  | 1/10       |
| IP                             | 10 <sup>-5</sup>            | 20M   | 0                                     | 0  | 0  | 1  | 0  | 1/20       |
|                                | 10 <sup>-4</sup>            | 10M   | 0                                     | 1  | 0  | 1  | 0  | 2/10       |
|                                |                             | 10F   | 0                                     | 1  | 0  | 1  | 0  | 2/10       |
|                                | 10 <sup>-3</sup>            | 10M   | 0                                     | 0  | 0  | 0  | 1  | 1/10       |
|                                |                             | 10F   | 1                                     | 0  | 0  | 0  | 0  | 1/10       |
|                                | 10 <sup>-2</sup>            | 10M   | 0                                     | 0  | 0  | 1  | 0  | 1/10       |
|                                |                             | 10F   | 0                                     | 0  | 0  | 0  | 0  | 0/10       |
| Control                        | -                           | 3M    | 0                                     | 1  | 0  | 0  | 0  | 1/3        |
|                                |                             | 12F   | 0                                     | 3  | 0  | 1  | 0  | 4/12       |

\* Males

\*\* Females

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TABLE 10

MORTALITY OF MICE RECEIVING BOTULINUM TOXIN  
FOLLOWING REMOVAL FROM FEED FOR 24 HOURS

| <u>Botulinum Toxin</u><br><u>Dilution</u> | <u>Death-Survivor Ratio</u> |                |                |
|---|-----------------------------|----------------|----------------|
|   | <u>Trial 1</u>              | <u>Trial 2</u> | <u>Trial 3</u> |
| 1:2                                       | 4/10                        | 9/10           | 3/10           |
| 1:4                                       | 3/10                        | 7/10           | 2/10           |
| 1:8                                       | 3/10                        | 5/10           | 2/10           |
| 1:16                                      | 0/10                        | 2/10           | 3/10           |
| 1:32                                      | 0/10                        | -              | 0/10           |
| 1:64                                      | 0/10                        | -              | 0/10           |

Following experiments to determine the potency of botulinum toxin by the stomach intubation route in fasted mice, tests were conducted in which mice intoxicated by this route were treated with antitoxin by the intraperitoneal route in order to determine if this treatment can indeed lessen the severity of symptoms or number of deaths. Three trials were conducted in which mice were inoculated by the stomach tube route and were injected by the intraperitoneal route at two hours with 0.1 ml of antitoxin (Wellcome Batch K 9710). The mice were observed daily for five days and deaths and/or surviving animals were recorded. The results of these studies are presented in Table 11.

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TABLE 11  
MORTALITY OF MICE INJECTED WITH BOTULINUM TOXIN  
AND TREATED INTRAPERITONEALLY WITH  
BOTULINAL ANTITOXIN

| Botulinum Toxin<br>Dilution | Death-Survivor Ratio |         |         |
|-----------------------------|----------------------|---------|---------|
|                             | Trial 1              | Trial 2 | Trial 3 |
| 10 <sup>-1</sup>            | 2/20                 | -       | -       |
| 10 <sup>-2</sup>            | 3/20                 | -       | -       |
| 10 <sup>-3</sup>            | 4/20                 | 4/20    | 4/10    |
| 10 <sup>-4</sup>            | 7/20                 | 2/20    | 1/10    |
| 10 <sup>-5</sup>            | -                    | 7/20    | 1/10    |
| 10 <sup>-6</sup>            | -                    | 10/20   | 1/10    |
| 10 <sup>-7</sup>            | -                    | -       | 2/10    |

Antisera therapy via this route appeared to be extremely variable and at the recommendation of the scientific technical representative, Dr. Carl LaManna, experiments in this area of study were terminated.

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In conducting the research described in this report, the Investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences, National Research Council.

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